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(54) Title: METHOD AND APPARATUS FOR THE MANIPULATION OF PARTICLES BY MEANS OF DIELECTROPHORESIS
(54) Titre: PROCEDE ET DISPOSITIF DE MANIPULATION DE PARTICULES PAR DIELECTROPHORESE

(57) Abstract

The invention relates to an apparatus and a method for establishing closed dielectrophoretic potential cages and precise displacement thereof, suitable for the manipulation of particles and detection of same. The apparatus comprises a first array of selectively addressable electrodes, lying on a substantially planar substrate and facing toward a second array comprising one electrode. The arrays define the upper and lower bounds of a micro-chamber where particles are placed in liquid suspension. By applying in-phase and counter-phase periodic signals to electrodes, one or more independent potential cages are established which cause particles to be attracted to or repelled from cages according to signal frequency and the dielectric characteristics of the particles and suspending medium. By properly applying voltage signal patterns into arrays, cages may trap one or more particles, thus permitting them to levitate steadily and/or move. In the preferred embodiment, where one array is integrated on a semiconductor substrate, displacement of particles can be monitored by embedded sensors to achieve complex operations upon the sample to be analyzed, such as isolation, selection and precise counting of particles.

(57) Abrégé

Cette invention concerne un dispositif et un procédé permettant d'établir des cages potentielles diélectrophorétiques fermées et leur déplacement précis, adapté à la manipulation de particules et à leur détection. Le dispositif comprend un premier réseau d'électrodes adressables de manière sélective, reposant sur un substrat essentiellement plan et faisant face à un second réseau comprenant une électrode. Les réseaux définissent les limites supérieures et inférieures d'un micro-compartiment dans lequel les particules sont placées en suspension liquide. L'application de signaux périodiques en phase ou en phase inverse aux électrodes permet d'établir une ou plusieurs cages potentielles indépendantes grâce auxquelles les particules sont attirées par ou repoussées des cages en fonction de la fréquence des signaux et des caractéristiques diélectriques des particules et du milieu de suspension. L'application de signaux de tension appropriés dans les réseaux permet aux cages de capturer une ou plusieurs particules, leurs permettant ainsi de léviter de façon régulière et/ou de se déplacer. Dans un mode de réalisation préféré dans lequel un réseau est intégré sur un substrat à semi-conducteur, le déplacement des particules peut être suivi par des détecteurs intégrés afin de réaliser des opérations complexes sur l'échantillon à analyser, telle que l'isolation, la sélection ou le comptage précis de particules.

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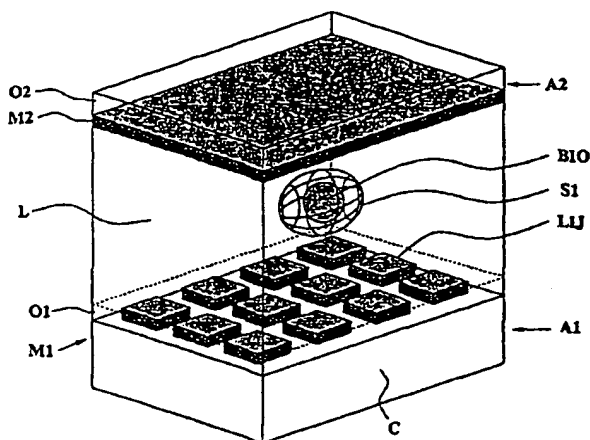
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(57) Abstract

The invention relates to an apparatus and a method for establishing closed dielectrophoretic potential cages and precise displacement thereof, suitable for the manipulation of particles and detection of same. The apparatus comprises a first array of selectively addressable electrodes, lying on a substantially planar substrate and facing toward a second array comprising one electrode. The arrays define the upper and lower bounds of a micro-chamber where particles are placed in liquid suspension. By applying in-phase and counter-phase periodic signals to electrodes, one or more independent potential cages are established which cause particles to be attracted to or repelled from cages according to signal frequency and the dielectric characteristics of the particles and suspending medium. By properly applying voltage signal patterns into arrays, cages may trap one or more particles, thus permitting them to levitate steadily and/or move. In the preferred embodiment, where one array is integrated on a semiconductor substrate, displacement of particles can be monitored by embedded sensors to achieve complex operations upon the sample to be analyzed, such as isolation, selection and precise counting of particles.



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Description

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METHOD AND APPARATUS FOR THE MANIPULATION OF PARTICLES BY MEANS OF DIELECTROPHORESIS

FIELD OF THE INVENTION

An apparatus and method are disclosed for the manipulation and detection of particles such as cells, polystyrene beads, bubbles, and organelles by means of dielectrophoretic forces.

BACKGROUND OF THE INVENTION

Dielectrophoresis (DEP) relates to the physical phenomenon whereby neutral particles, when subject to nonuniform, time stationary (DC) or time varying (AC) electric fields, experience a net force directed towards locations with increasing (pDEP) or decreasing (nDEP) field intensity. If the intensity of the said dielectrophoretic force is comparable to the gravitational one, an equilibrium may be established in order to levitate small particles. The intensity of the dielectrophoretic force, as well as its direction, strongly depend on the dielectric and conductive properties of particles and on the medium in which the body is immersed. In turn, these properties may vary as a function of frequency for AC fields.

A description of the theory of dielectrophoresis has been published by H. A. Pohl in "Dielectrophoresis" Cambridge University Press (Cambridge 1978). A theoretical formulation of a case of particular interest is reported in *Biochimica et Biophysica Acta* 1243 (1995) p. 185-194, and *Journal of Physics, D: Applied Physics*, 27 (1994) pp. 1571-1574.

Studies on the action of dielectrophoresis on both biological matter (cells, bacteria, viruses DNA, etc.) and inorganic matter particles have lately proposed using DEP forces for the isolation of elements from a mixture of microorganisms, their

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5 characterization by differences in physical properties and their general manipula-
tion. For such purposes, the suggestion has been to utilize systems of the same
scale of particle size, in order to reduce the potentials required by electrical field
10 distributions.

U.S. Pat. 5,888,370, U.S. Pat. 4,305,797, U.S. Pat. 5,454,472, U.S. Pat. 4,326,934,
15 U.S. Pat. 5,489,506, U.S. Pat. 5,589,047, U.S. Pat. 5,814,200, teach different meth-
ods of separating particles in a sample, based on differences in dielectric and con-
ductive properties characterizing the species they belong to. The main drawback,
common to all devices proposed resides in the requirement of mechanical and fluid
20 dynamic microsystems for moving fluids within the system. Moreover, each appa-
ratus of the above listed patents involves contact and friction of particles with the
surfaces of the system, compromising their mobility and integrity.

25 U.S. Pat. 5,344,535 teaches a system for the characterization of microorganism
properties. The disclosed apparatus and the proposed method have the shortcoming
of providing data on a large number of bodies, lacking the advantages of analysis on
30 a single particle. In addition, the disclosed system is unable to prevent contact of
particles with device surfaces.

U.S. Pat. 4,956,065 teaches an apparatus to levitate single particles and an-
35 alyze their physical properties. However, this device requires a feedback control
system since it employs pDEP. Moreover, the system is unsuitable for miniaturiza-
tion, having a three-dimensional topology which is not compatible with mainstream
40 microelectronic fabrication technologies.

The paper by T. Schnelle, R. Hagedorn, G. Fuhr, S. Fiedler, T. Muller in
"Biochimica et Biophysica Acta", 1157(1993) pp. 127-140, describes research and
45 experiments on the creation of three-dimensional potential cages for the manipula-
tion of particles. However, the proposed structures are very difficult to fabricate in
scale with the size of cells (required for trapping a single cell in the cage). In fact,
50

5 the major problem of these systems is the vertical alignment of two structures on a micro-metric scale.

10 SUMMARY OF THE INVENTION

The present invention relates to a method for the stable levitation and independent
15 motion of neutral particles in a liquid suspending medium and their precise displacement by means of an electronically programmable device adapted to receive such a solution.

20 As used above, the term "particle" is intended to include biological matter such as cells, cell aggregates, cell organelles, bacteria, viruses and nucleic acids as well as inorganic matter such as minerals, crystals, synthetic particles and gas bubbles. By "dielectrophoretic potential" what is meant is a three-dimensional (3D) scalar
25 function whose gradient is equal to the dielectrophoretic force. By "equipotential surface" what is meant is a surface defined in the 3D space whose points have the same dielectrophoretic potential; the dielectrophoretic force is always perpendicular
30 to said surface. By "potential cage" what is meant is a portion of space enclosed by an equipotential surface and containing a local minimum of the dielectrophoretic potential. By "particle trapped inside a potential cage" what is meant is a particle
35 subject to dielectrophoretic force and located inside the said cage. At equilibrium, if the particle is subject to dielectrophoretic force only, then it will be located at a position corresponding to the said dielectrophoretic potential minimum, otherwise
40 it will be positioned at a displacement from that minimum given by the balance of forces.

45 The preferred, but not exclusive, embodiment of the present invention, comprises two main opposed modules; the first one comprises a plurality of electrically conductive electrodes, whose shape may be of various types, regularly arranged on a
50 insulating substrate; the electrodes may be optionally coated with an insulating layer

5 protecting them from charge carriers present in the liquid suspension. If this module is realized with integrated circuit fabrication technology, it may include memory
10 elements for electrode programming, configurable signal generators such as sine or square wave, impulse etc., with variable frequency and phase, any integrable sensor device for detecting the presence of the particle, input/output circuits etc.. The second module comprises a single large electrode fabricated in a conductive, optionally
15 transparent matter, which in turn may be coated with an insulating layer. It is to be understood that this large electrode may also be split into several electrodes, if desired. A spacer can be inserted between the first (lower) module and the second
20 (upper) one in order to implement a chamber for the containment of the sample to be analyzed or manipulated. The same spacer may also serve to establish separation walls inside the device so as to realize multiple chambers. Of course, the spacer may
25 also be integrated in either the first or second module, or both. Finally, a visual inspection system such as a microscope and camera may be added to the device, as well as fluidics systems for moving liquid or semi-liquid matter in and out of the
30 device.

The architecture of the apparatus described allows one, by simply applying in-phase and counter-phase periodic signals to the electrodes, to establish in the micro-
35 chamber one or more independent potential cages, the strength of which may be varied by acting on the frequency as well as on the amplitude of the signals applied. The cages may trap one or more particles, thus permitting them either to levitate
40 steadily or to move within the micro-chamber, or both. Due to this feature, any contact or friction of the particles with the chamber borders and the electrodes can be avoided. The height and relative displacement of cages can be independently set
45 by an appropriate choice of signals and does not require any mechanical adjustment. Thus, the device can be configured as a fully programmable electronic apparatus.

The methodology for the displacement of the potential cage along the micro-
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5 chamber is much like the principle used in charge coupled devices (CCDs). For
example, if a first electrode is in-phase with the upper module and is surrounded by
10 electrodes connected to counter-phase signals, a potential cage is established on top
of it. Then, by simply applying in-phase signals to one of the adjacent electrodes
(in the same direction as the programmed motion) the potential cage spreads over
15 the two electrodes thus aligning its center in between them: the particle has thus
moved half of the cell-pitch. Once the transient has expired the phase is reversed
for the first electrode (where the particle was located at the beginning of the phase):
20 this causes the potential cage to shrink and to move on top of the in-phase electrode
which is displaced one cell-pitch away from the previous electrode. By repeating the
latter operation along other axis any potential cage may be moved around the array
plane.

25 The shortcomings of devices known from the prior art can be overcome thanks
to the apparatus according to the present invention, which allows one to establish
a spatial distribution of electric fields that induce closed dielectrophoretic potential
30 cages. The proposed device does not require precise alignment of the two main mod-
ules, thus optimizing both simplicity and production cost: it overcomes most of the
restrictions related to the implementation cost and to the minimum allowable cage
35 potential size inherent in the prior art (alignment gets more and more critical as
the electrode size shrinks). Hence misalignment of the two main modules does not
compromise the system functionality. The importance of this feature may be bet-
40 ter appreciated if one thinks of all the applications in which the device is manually
opened and/or closed, requiring repeated and flexible use; it may thus be imple-
mented in low-cost, standard manufacturing microelectronic technology. Moreover,
45 the proposed device easily allows trapped particles to be displaced along a wide range
compared to the particle size.

50 In addition, no prior art system that employs fluidics or "traveling fields" for the

5 displacement of particles achieves precise particle positioning while keeping particles
away from device surfaces; yet, it is apparent that such a result can be achieved
10 if three-dimensional potential-cages positioned at a fixed height and movable along
other directions of the apparatus are available. Further advantages of the invention
stem from the possibility to control the height of the cage potentials by adjusting
15 the voltage values applied.

Thanks to the flexible programming of the disclosed invention, virtual paths can
be established, thus avoiding the need for application-specific devices and widening
20 the range of potential applications and users. Furthermore, the ability to integrate
optical and/or capacitive sensing allows one to overcome the need for bulky detec-
tion instrumentation normally used in this field, such as microscopes and cameras,
although it does not prevent it from being used for visual inspection of the internal
25 micro-chamber. Processing the integrated sensors information with feedback control
techniques, enables complex operations to be carried out in a fully automated way:
for example, characterization of the physical properties of particles under test.

30 Finally, the closed potential cage approach prevents particles from getting out of
control in the presence of: hydrodynamic flows due to thermal gradients, significant
Brownian motions (equally likely from any direction), or forces due to Archimedes'
35 balance. In fact, in all the above cases, any apparatus providing non-closed potential
surfaces proves ineffective, since it cannot counterbalance upward forces.

40 Some unique features of the apparatus according to the present invention, as
compared to those present in the prior art, may be summarized as:

1. the capability of establishing closed dielectrophoretic potential cages without
45 requirements of alignment between modules, whereby single or groups of par-
ticles are independently trapped in the cages and placed in stable suspension
by means of dielectrophoretic forces without any friction with electrodes or
50 boundaries.

- 5
2. The ability to move any potential cage independently around the micro-chamber by virtue of electronically programmed electric signals.
- 10
3. The possibility of shrinking the cage size according to application requirements and implementation, thus permitting fabrication of the device in microelectronic technology with implementation of embedded sensors, actuators and
- 15
- signal generation.

BRIEF DESCRIPTION OF THE DRAWINGS

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FIG. 1 shows a schematic three-dimensional view of a part of the device devoted to sample manipulation, with the modular structure formed by the substrate, including the electrodes, and the lid;

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FIG. 2 shows a detailed cross-sectional view of the same structure as in FIG. 1;

FIG. 3 shows an embodiment of the electrode arrangement ;

FIG. 4 shows an alternative embodiment of the electrode arrangement;

30

FIG. 5 shows a blow-up schematic diagram of the device emphasizing the presence of a third module;

FIG. 6 shows a three-dimensional surface in which each point has the same root mean square (RMS) electric-field magnitude;

35

FIG. 7 shows the same plot as in FIG. 6 for a different set of signals applied;

FIG. 8 sketches the cage motion principle highlighting the fundamental steps and their timing;

40

FIG. 9 shows a 2-D plot of the RMS magnitude of the electric field on a vertical section orthogonal to the electrodes, assuming that electrodes extend for the whole device length;

45

FIG. 10 shows the same plot as in FIG. 9 for a different set of voltages applied;

50

5 FIG. 11 shows a plot of the absolute value of the gradient of the square RMS
magnitude of the electric field along a horizontal cross section of the plot in FIG. 9
10 passing through the dielectrophoretic potential minimum ($4.3\mu\text{m}$ above the electrode
surface);

15 FIG. 12 shows a plot of the absolute value of the gradient of the square RMS
magnitude of the electric field, along a vertical section of the plot in FIG. 9 passing
through the dielectrophoretic potential minimum for different values of the voltage
applied to the upper electrode;

20 FIG. 13 shows a plot of the absolute value of the gradient of the square RMS
magnitude of the electric field, along an horizontal cross section of the plot in FIG.
10 passing through the dielectrophoretic potential minimum;

25 FIG. 14 shows a plot of the absolute value of the gradient of the square RMS
magnitude of the electric field, along a vertical section of the plot in FIG. 10 passing
through the dielectrophoretic potential minimum;

30 FIG. 15 shows a simplified block diagram of the first substrate;

FIG. 16 sketches the block diagram of a cell in the array;

35 FIG. 17 sketches the measurement instruments which may be interfaced with the
apparatus;

40 FIG. 18 shows a schematic plot of the nDEP potential along a generic section,
comparing cage size with particle one;

45 FIG. 19 sketches a special electrode layout which enables one to optimize the
area available for the electrode programming circuit;

50 FIG. 20 sketches a special electrode layout which allows for optimization of the
area available for the electrode circuitry relating to a specific embodiment targeted
to particle counting;

55 FIG. 21 shows an embodiment of an integrated optical sensor;

FIG. 22 shows an embodiment of an integrated capacitive sensor;

FIG. 23 shows an embodiment of an integrated capacitive sensor;

DETAILED DESCRIPTION

The features and advantages of the invention will be clearer from the description of embodiments illustrated by examples in what follows. It is to be understood that examples used herein are for purpose of describing a particular embodiment and are not intended to be limiting of the spirit of the invention.

Dielectrophoretic potential energy

A dielectric sphere immersed in a liquid at coordinates (x, y, z) , and subject to the effect of spatially non-uniform AC or DC electric fields, is subject to a dielectrophoretic force $F(t)$ whose time-averaged value is described by the following:

$$\begin{aligned} \langle F(t) \rangle = 2\pi\epsilon_0\epsilon_m r^3 \{ & \text{Re}[f_{CM}] \nabla (E_{RMS})^2 + \\ & + \text{Im}[f_{CM}] (E_{x0}^2 \nabla \varphi_x + E_{y0}^2 \nabla \varphi_y + E_{z0}^2 \nabla \varphi_z) \} \end{aligned} \quad (1)$$

where ϵ_0 is the vacuum dielectric constant, r is the particle radius, E_{RMS} is the root mean square value of the electric field, E_{x0}, E_{y0}, E_{z0} are the electric field component along axes x, y, z , while $\varphi_{x,y,z}$ are the phases of the electric field component and f_{CM} is the well known Clausius-Mossotti factor defined as:

$$f_{CM} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}$$

where ϵ_p^* and ϵ_m^* represent the relative complex permittivity of the particle and of the suspending medium respectively, defined as: $\epsilon_{m,p}^* = \epsilon_{m,p} - i\sigma/(\epsilon_0\omega)$, where ϵ is the relative dielectric constant, σ is the conductivity, ω is the angular frequency and i is the square root of minus one.

If electric field phases are constant, equation (1) may be simplified to:

$$\langle F(t) \rangle = 2\pi\epsilon_0\epsilon_m r^3 \text{Re}[f_{CM}] \nabla(E_{RMS})^2 \quad (2)$$

where nDEP is defined by $\text{Re}[f_{CM}] < 0$ while pDEP is defined by $\text{Re}[f_{CM}] > 0$. For high values of ω , where $\epsilon_m^*, \epsilon_p^* \leftarrow \epsilon_m, \epsilon_p$ pDEP is established on a particle whenever $\epsilon_m < \epsilon_p$ whilst nDEP is established whenever $\epsilon_m > \epsilon_p$. Since $\epsilon_{m,p}^* = \epsilon_{m,p}^*(\omega)$, thus $f_{CM} = f_{CM}(\omega)$ so that $\text{Re}[f_{CM}]$ may have different signs for different species of particle at a given frequency. The method of choosing an angular frequency ω so that two different species of particles experience nDEP and pDEP respectively, is commonly used as known art for selection purposes.

Since the force described in equation (2) is conservative, it is possible to define the dielectrophoretic potential energy:

$$\langle W \rangle = -2\pi\epsilon_0\epsilon_m r^3 \text{Re}[f_{CM}] (E_{RMS})^2,$$

where,

$$\langle F(t) \rangle = -\nabla \langle W \rangle,$$

If the voltage signals applied to electrodes and establishing the electric field are periodic, it can easily be shown that

$$\langle W \rangle = -\alpha 2\pi\epsilon_0\epsilon_m r^3 \text{Re}[f_{CM}] E^2 \quad (3)$$

where α is a constant that depends on the shape of the voltage signals applied to electrodes and E is the magnitude of the electric field, (e.g. $\alpha = 1$ for square-wave signals and $\alpha = 1/\sqrt{2}$ for sinusoidal signals). Thus, minima of E^2 are also minima of the negative dielectrophoretic potential (since for nDEP, $\text{Re}[f_{CM}] < 0$) as well as

maxima of the positive dielectrophoretic potential (since for pDEP, $\text{Re}[f_{CM}] > 0$). In what follows, "dielectrophoretic potential" will be used as a synonym of "negative dielectrophoretic potential". Furthermore, since E^2 is a monotonic function of E , the minima or maxima of E correspond to the minima or maxima of the dielectrophoretic potential function $\langle W \rangle$. This is very useful since the location of the dielectrophoretic potential minima or maxima can be found by time-stationary simulations of the electric field as illustrated by the figures enclosed. To summarize the above concept, it can be easily demonstrated that:

any dielectrophoretic potential cage (containing nDEP potential energy local minima) is enclosed by at least one imaginary closed surface composed of points of the space having constant electric field magnitude.

If the spherical and homogeneous particle is subject to the gravitational force:

$$F_g = \frac{4}{3}\pi R^3 \Delta\rho g$$

where $\Delta\rho$ is the mass density difference between the particle and the medium and g is the acceleration of gravity (9.807 m/s^2), as well as to nDEP, then stable suspension is achieved according to:

$$\langle F(t) \rangle > F_g. \quad (4)$$

Since the relative dielectric constant cannot be greater than unity (e.g. if the particle is a bubble of air immersed in water, where $\epsilon_p = 1$ and $\epsilon_m \simeq 81$), then the minimum value of ∇E_{rms}^2 required for balancing the gravitational force acting on the particle can be estimated, by using equation (4), as $1.835 \cdot 10^3 (V/cm)^2 / \mu m$ which is achievable by using standard microelectronic technology and/or micro-machining techniques. Again, particles that are twice as heavy than water ($\Delta\rho \simeq 1000 \text{ Kg/m}^3$)

5 can be suspended in water, if the relative dielectric constant of the medium is at least $2.2 \div 20.3$ times greater than that of the particle for typical values of ∇E_{rm}^2 .

10 General structure of the device

The apparatus according to the preferred embodiment comprises two main modules. The first module A1 (FIG. 1) comprises an array M1 of selectively addressable electrodes LIJ (FIG. 1 and 2) being disposed upon an insulating substrate O1, grown on a semiconductor substrate C (FIG. 1 and 2). The second module A2 is made up of a single large electrode M2 which is fabricated on a substrate O2 (FIG. 1 and 2) and is opposed to the said array M1. In between the two modules a micro-chamber (L in FIG. 1 and 2) is formed, containing the particles (BIO in FIG. 1) in liquid suspension. Methods for containing the liquid suspension in the micro-chamber will be described later on. The first module A1 is made in silicon, according to known microelectronic technology, or any other suitable substrate materials, such as glass, silicon dioxide, plastic, or ceramic materials. An electrode may be of any size, preferably ranging from sub-micron ($\sim 0.1\mu m$) to several millimeters (mm) with $5\mu m$ to $100\mu m$ being the preferred size range for devices fabricated using micro-lithographic techniques, and $100\mu m$ to $5mm$ for devices fabricated using micro-machining and/or printed circuit board (PCB) techniques. The device can be designed to have as few as under ten electrodes or as many as thousands or millions of electrodes. The distance DL between the two modules may vary according to the embodiments but is preferably in the order of magnitude of the electrode size DE (FIG. 2).

Electrodes can be coated by an insulating layer (R1 in FIG. 2) to prevent electrolysis due to the interaction of electrodes with the liquid medium, which may contain a high concentration of positive and negative ions. Such a layer may be avoided if either the electrodes are composed of material that does not chemically react with the liquid medium or the frequency of signals energizing electrodes is high enough

5 to make electrolysis negligible. Finally, some circuitry, the purpose of which will be explained later in greater detail, may be placed underneath each electrode.

10 Array electrodes may be of any shape, depending on the effect to be achieved; for example's sake, an array M1 of square electrodes are shown in the preferred embodiment of FIG. 1, while FIG. 2 shows a cross-section of electrodes emphasizing their width and relative displacements (DE and DO).

15 In an alternative embodiment, electrodes may be of hexagonal shape (as illustrated in FIG. 3), which allows the number of electrodes to establish a single potential cage to be reduced from 9 to 7 (as will be shown later) and offers a larger number of possible cage motion directions DIR (from 4 to 6).

20 The second main module A2 comprises a single large electrically conductive electrode (M2 in FIG. 1 and 2) which is opposed to the first module A1. It also serves as the upper bound of chamber L containing the liquid suspension of particles. This electrode may be coated with an insulating layer (R2 in FIG. 2) to protect it against electrolysis and may have a mechanical support (O2 in FIG. 1 and 2). In the preferred embodiment, this electrode is a single, planar surface of conductive glass, thus permitting visual inspection of the micro-chamber.

25 A spacer A3 (FIG. 5) is used to separate the two modules (A1 and A2 in FIG. 5, in which A1 comprises R1, O1, M1 and C, while A2 comprises R2, O2, M2) by a given distance (DL in FIG. 2). The spacer may also be used to contain the sample for manipulation or analysis.

30 By applying appropriate time-varying signals to different subsets of electrodes, a potential cage S1 (FIG. 1 and FIG. 6) that may contain one or more particle BIO is established upon one or more electrode. The potential cage is located at some height above the array plane, the value of which depends on the signals applied, on the ratio of electrode size DE and pitch DO and on the distance between the two modules DL. By changing the subset of electrodes to which signals are applied,

one or more potential cages may be moved around micro-chamber L in a direction parallel to the electrode array.

From simulation results, emerges that, for constant values of size DL, the greater the ratio between size DE and DO, the better the properties of the cage in terms of DEP force strength.

Method for establishing potential cages

In order to establish potential cages on top of a single electrode, a pattern of voltage signals is applied to corresponding subsets of electrodes. FIG. 4 illustrates a set of electrodes L1-L12 in array M1, used as a reference for numerical simulations.

Defining:

$$V_{sq}(\omega t, \varphi) = \begin{cases} 1 & \text{if } \cos(\omega t + \varphi) > 0 \\ -1 & \text{if } \cos(\omega t + \varphi) \leq 0 \end{cases}$$

as a square wave signal having period T , where $\omega = 2\pi/T$, the following voltage signals are applied to electrodes:

$$V_{La} = V_e \cdot V_{sq}(\omega t, \varphi) \quad \forall a \in \{1-6, 8-12\}$$

$$V_{L7} = V_e \cdot V_{sq}(\omega t, \varphi + \pi)$$

$$V_{M2} = V_c \cdot V_{sq}(\omega t, \varphi + \pi)$$

where V_{La} , $a \in \{1-12\}$ are signals applied to electrodes L1-L12, V_{M2} is the voltage signal applied to M2, and V_e and V_c are constant values. Using voltage

patterns as indicated above, the electric field phases are constant, so that equation (2) applies. Hence, the numerical simulations of the electric field magnitude will be used to verify the establishing of dielectrophoretic potential cages.

FIG. 6 shows the result of a numerical simulation regarding the same set of electrodes as illustrated in FIG. 4 energized by the above mentioned voltage signal patterns where: $DE = 5\mu m$, $DO = 1\mu m$, $DL = 10\mu m$, $V_e = 2.5V$, $V_c = 0V$. Water is chosen as the liquid medium between the modules A1 and A2, with $\epsilon_m \simeq 81$. $R2$ is negligible and $R1 = 1\mu m$. The plot in FIG. 6 shows a 3D environment containing a closed surface whose points are characterized by having a constant electric field magnitude (S1 in FIG. 6) at $400V/cm$. This proves, by virtue of equation (3), that the dielectrophoretic equipotential surface is likewise closed, hence a potential cage is established on top of L7. Thus, a pattern of only two signals, having the same frequency and counter-phase relationship, is needed to establish a minimum of the dielectrophoretic potential function on top of L7. From simulation it also emerges that by increasing $V_c \in [-2.5, 2.5]V$ the dielectrophoretic forces of the cage increase, while the cage height decreases with respect to the array plane. In the preferred embodiment, in which square electrodes are employed, the minimum number of array electrodes for establishing a single dielectrophoretic potential cage is 9 (L2-L4, L6-L8, L10-L12 in FIG. 4). On the other hand, if a hexagonal array of electrodes is employed, as illustrated in FIG. 3, the minimum number of array electrodes for establishing a single dielectrophoretic potential cage is 7, such as electrodes E1-E7.

In order to establish potential cages at a mid point on top of two electrodes, a different pattern of voltage signals is applied to corresponding subsets of electrodes. FIG. 7 shows the result obtained when the stimuli applied to the electrodes are as follows:

$$V_{La} = V_e \cdot V_{sq}(\omega t, \varphi) \quad \forall a \in \{1 - 5, 8 - 12\}$$

$$V_{L6} = V_{L7} = V_c \cdot V_{sq}(\omega t, \varphi + \pi)$$

$$V_{M2} = V_c \cdot V_{sq}(\omega t, \varphi + \pi),$$

where all the other parameters are the same as before. S2 in FIG. 7 again shows a closed surface whose points have a constant electric field strength at 400V/cm, where the center is, however, located on top of the mid point between electrodes L6 and L7.

This last pattern of voltage signals, in combination with the previous one, can be used for moving potential cages in a programmed direction. More specifically, by repeatedly changing the subsets of electrodes to which in-phase and counter-phase signals are respectively applied, in particular by alternating and shifting the two patterns described in a given direction, it is possible to move the potential cage in that direction. As an example, FIG. 8 sketches three plots where the potential cage is moved from a position on top of L7 to another position on top of L6: the first at time T1, the second at T2 and the third at T3. In each plot the phase of electrodes L5, L6, L7, L8 is reported, showing the moving-cage principle. With increasing time, the electrode with phase $\varphi + \pi$ shifts along a decreasing X direction in two steps: at T2 electrode L6 is connected to a signal having phase $\varphi + \pi$ which is the same as L7 and then, at time step T3, the phase of L7 is reversed.

Obviously, the time interval between switching phases should be carefully chosen according to system characteristics: force intensity, fluid medium viscosity, particle size, etc.. For this purpose it may be useful to employ embedded sensors to detect the presence/absence of one or more particles in each position so that the time distance

can be adjusted according to sensor data.

To illustrate the capability of the invention to move closed dielectrophoretic cages, FIG. 9 and 10 show 2-D simulations of the electric field distribution along a cross section of the device. When the voltages applied to electrodes P1, P2 and P3, and the lid electrode M2 are:

$$V_{Pa} = V_e \cdot V_{sq}(\omega t, \varphi) \quad \forall a \in \{1, 3\}$$

$$V_{P2} = V_e \cdot V_{sq}(\omega t, \varphi + \pi)$$

$$V_{M2} = V_c \cdot V_{sq}(\omega t, \varphi + \pi)$$

where, $V_e = 2.5V$ and $V_c = 0$, the resulting electric-field distribution is as shown in FIG. 9, in which the darker regions S3 mean a lower electric-field magnitude, while the brighter regions mean a higher electric-field magnitude.

FIG. 11 shows a plot (in log scale) of the absolute value of the gradient of the square electric field magnitude, taken along a horizontal cross section of the plot of FIG. 9 passing through the center of the cage ($4.3\mu m$ above the array surface). This kind of plot is very useful since the values of the plots are directly proportional to the dielectrophoretic force, from which one can pinpoint the location of the minimum dielectrophoretic potential (where dielectrophoretic forces are equal to zero). FIG. 12 shows a similar plot taken along a vertical cross section of the plot of FIG. 9 including the center of the potential cage for different values of V_c , ranging from $+2.5V$ to $-0.5V$.

In order to establish a dielectrophoretic potential cage in the region above the mid point between P2 and P3, the following voltages can be applied:

$$V_{P1} = V_a \cdot V_{sq}(\omega t, \varphi)$$

$$V_{P2} = V_{P3} = V_e \cdot V_{sq}(\omega t, \varphi + \pi)$$

$$V_{M2} = V_c \cdot V_{sq}(\omega t, \varphi + \pi)$$

where $V_e = 2.5V$ and $V_c = 1.5V$. The result is shown in FIG. 10 where S4 is the region in which the potential cage is located.

FIG. 13 shows a plot of the absolute value of the gradient of the square electric field magnitude, along a horizontal cross section of the plot in FIG. 10 including the cage center, in the case of $V_e = 1.5V$; the height of the cage center from the array surface is $4.3\mu m$. The presence of two values with gradient equal to zero in FIG. 13 is due to a maximum on top of electrode P1 and to a minimum located in the region above the mid point between P2 and P3. A given particle subject to such a dielectrophoretic force field would find a stable equilibrium point at the aforesaid minimum and an unstable equilibrium point at the aforesaid maximum. FIG. 14 shows a similar plot taken along a vertical cross section of the plot of FIG. 10 passing through the cage center, in the case of $V_e = 1.5V$.

To summarize, the establishing of dielectrophoretic potential cages, as disclosed by the present invention, can be achieved by using a pattern of as few as two voltage signal having the same frequency and counter-phase relationship. Furthermore, movement of such cages along a guide path parallel to the array surface can be achieved by simply selecting convenient patterns of subsets of electrodes to which

5 apply the two above mentioned signals at different time steps. The electrode voltage waveforms may either come from on-chip oscillators or from external generators.

10 **Preferred embodiment: integration on semiconductor substrate**

A schematic diagram of the first module A1 in the preferred embodiment is illustrated in FIG. 15. A silicon substrate embeds an array M3 of micro-locations EIJ
15 that are independently addressed by proper addressing circuits, DX e DY, by means of a number of electrical communication channels running along vertical lines YJ and horizontal lines XI. The module communicates with external signals XYN by means
20 of an interface circuit IO, which in turn communicates by means of connection CX and CY with addressing circuits DX e DY, and by means of a set of connections CS controls the waveform generation and sensor readout circuit DS for delivering
25 the signal to be applied to the micro-locations EIJ and for collecting signals from the sensors in the micro-locations by means of connections FS. The apparatus is connected with a number of fluidic communication channels FM with the external
30 means IS for the management of liquid suspension medium containing the particles. Various instruments can be used for interfacing to the device SS by means of electrical communication channels XYN such as: computer, external waveform generators,
35 analyzers etc. (WS in FIG. 17), and by means of fluidic dynamic channels, such as micro-pumps IS and by means of optical channels OC such as microscope, camera, etc. MS.

40 In the preferred embodiment each micro-location EIJ (FIG. 16) comprises at least one electrode LIJ to be energized by the electrical signals, a circuit for the electrode signal management MIJ (FIG. 16) and a sensor SIJ to detect the pres-
45 ence/absence of particles on top of each cell. Each of these blocks may communicate with others inside the same element by means of local connections C1, C2, C3. Moreover the circuit for electrode signal management (MIJ FIG. 16) can communi-
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5 cate with external circuits by means of global connections **XI** and **YJ**. The circuit
10 **MIJ** may contain switches and memory elements suitable for selecting and storing
the routing of pattern signals to electrode **LIJ**. Since two voltage signal patterns
are sufficient for establishing and moving dielectrophoretic potential cages, as ex-
15 plained in the previous section, one electronic memory means is sufficient to deter-
mine whether the electrode will be connected to the in-phase or to the counter-phase
signal. To optimize the space available, various different arrangements of **LIJ**, **SIJ**
and **MIJ** are possible: for example **LIJ** may entirely overlap **MIJ** and partially cover
20 **SIJ** or simply be placed beside **SIJ** according to the microelectronic technology rules.

A peculiar characteristic of the present invention considered to be unique from
prior art dielectrophoretic devices, consists in its ability to integrate on the same
25 substrate both actuators, for biological particle manipulation, and sensors for detec-
tion of particles. Some indicative but not exclusive examples of integrated sensors
are shown in FIG. 21, 22 and 23.

FIG. 21 sketches an implementation of a sensing scheme using an optical sensor
30 to detect the presence/absence of a biological particle **BIO**. If the lid **A1** is made of
transparent and conductive material, a window **WI** can be opened on the electrode
LIJ. The size of **WI** is negligible for modifying the dielectrophoretic potential but
35 large enough to permit a sufficient amount of radiation to impinge onto the sub-
strate. Underneath **LIJ** a photo-junction **CPH** working in continuous or storage
mode is realized into substrate **C** according to known art. The presence/absence of
40 the biological element **BIO** determines the amount of optical energy reaching the
photodiode, causing a change of charge accumulated across **CPH** during the inte-
gration time. This variation is detected by a conventional charge amplifier **CHA**
45 composed of an amplifier **OPA**, a feedback capacitor **CR** and a reference voltage
source **VRE**. The connection to this charge amplifier is established by enabling a
switch **SW1** after switch **SW2** has been opened, thus permitting the accumulated
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5 charge to be integrated onto CR. The photodiode and charge amplifier are designed,
according to known art, to obtain a signal to noise ratio sufficient to detect the pres-
10 ence/absence of the biological particle. As an example, with reference to a structure
with the dimensions previously described for simulations, and assuming a $0.7\mu\text{m}$
CMOS technology, we may consider a photodiode of $1 \times 2\mu\text{m}$ in the substrate under
the electrode. Analyzing the signal to noise ratio according to known art, a varia-
15 tion of 10% of the particle transparency with respect to the liquid medium can be
revealed using integration times larger than $3\mu\text{s}$.

20 In another embodiment, capacitive sensing is used as sketched in FIG. 22. A
voltage signal SIG applied to the lid A1 induces a variation in the electric field ELE
between A1 and LIJ. The corresponding capacitance variation can be detected by
a charge amplifier CHA similar to the case of optical sensing.

25 In FIG. 23 another implementation of capacitive sensing is sketched, using two
electrodes FR1 and FR2 coplanar to element LIJ. A voltage signal SIG applied to
the element FR1 determines a variation in the fringing electric field ELE towards
30 FR2. The interposition of biological element BIO in the region affected by this
electric field causes a variation in the capacitance value between FR1 and FR2.
This variation is detected by a charge amplifier CHA similar to the previous sensing
35 schemes. The electrodes FR1 and FR2 may be omitted if the elements LIJ of the
adjacent locations are used in their place. It is to be understood that more than one
of the above described sensing principles may be used in the same device to enhance
40 selectivity. As an example, different particles having the same transmissivity but
a different dielectric constant, or having the same dielectric constant and different
transmissivity may be discerned, by using a combination of capacitive and optical
45 sensors.

50 An outstanding feature believed to be characteristic of the present invention is the

5 possibility to isolate single microorganisms of a size within the micron or sub-micron
range, and to do so on a large number of them; indeed the size of microorganism
10 which can be isolated will shrink following the advances in standard microelectronic
fabrication technologies, in line with the shrinking in the minimum feature sizes that
is characteristic of the technology. Indeed, if the size of the dielectrophoretic potential
15 cage is small enough, no more than one particle of a given size may be trapped inside
the cage. In order to better understand this feature of the device one can consider
the distribution of the dielectrophoretic potential P (FIG. 18) along a horizontal
20 cross section passing through the center of the cage, as established by the method
disclosed, which has the typical behavior shown in FIG. 18 where two local maxima
represent the borders of the cage potential along direction X . If the relative distance
25 DP is twice the particle radius R to be isolated, then only one of the particles of the
neighborhood will find room in the cage, so that if the cage is already occupied by
a particle, an outward net force is exerted on other candidate particles, thus moving
30 excess particles into either empty neighborhood cages or lateral reservoirs designed
to contain the overspill particles. It is to be noted that if the above operation needs
to be applied to all particles of the sample, the particle density should be smaller
than the cage density.

35 The dielectrophoretic cage size is solely limited by the area dedicated to the
circuitry of each electrode, which in turn depends on the technology adopted. To
overcome this limit, a different electrode arrangement may be used, as disclosed in
40 what follows, in which alternative electrode topologies are employed that are less
flexible but more optimized with respect to potential cage size and targeted to appli-
cations requiring greater sensitivity such as sub-micron microorganism manipulation
45 and counting. For applications requiring potential cages smaller than the area needed
by electrode circuitry, alternative embodiments may be employed in order to achieve
better area optimization.

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As an example, in order to increase the area available for circuitry by 25%, it is feasible, using the same arrangement of electrodes, to connect an electrode LN (FIG. 19) out of a cluster of four LL to a fixed voltage signal pattern (for example to the in-phase one). From now on, we will refer to electrodes of type LN as "non-programmable electrodes" since they cannot be switched among the various voltage signal patterns but are tied to a fixed one. The above embodiment has the shortcoming of restricting the motion of potential cages solely along guide paths DR. On the other hand, the electrode arrangement shows the advantage of saving area for circuitry due to the fact that MIJ and SIJ blocks are not implemented in non-programmable electrodes LN.

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Another alternative embodiment which further exploits the method for shrinking cage size at the expense of device flexibility is disclosed in FIG. 20. In this case the direction of motion is reduced to one dimension, along guide paths DR, and the cells SI (FIG. 20), designed for sensing the presence and possibly the type of particles, are arranged along one column SC, orthogonal to the allowed motion direction. Using proper signals, potential cages are regularly established along rows and moved along the guide paths DR throughout the column SC into a chamber CB designed to contain the particles whose number (and possibly type) has already been detected. Since motion directions along vertical guide paths are not used, non programmable electrodes LN are floor planned to save area available for cell circuitry. Hence, the area available for cell circuitry and for sensors is optimized since only one electrode in two needs to be programmed, and only cells SI need to integrate a sensor. The main shortcoming of this last alternative embodiment as compared to the preferred one resides in the longer time required for detecting the particles in the sample, since it depends on the number of row cells that particles must step through before reaching the sensors. On the other hand, the latter alternative embodiment can achieve smaller cage size, thus counting smaller particles.

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Another approach according to the present invention is that of estimating the number of particles smaller than feasible cage size by taking advantage of sensors whose output is proportional to the number of particles contained into a cage. In using this method, cage size does not need to be set to minimum since the total number of particles can be estimated by summing the number of them in each cage, even if the the latter contain a plurality of particles. The main drawback of this approach is that the output of the sensors is designed to depend only on the number of particles, regardless of their type, so that their type cannot be detected.

Once the sample is inserted into the device -by means and instruments known to those with ordinary skill in the art such as micro-pump syringes etc., in fully automated or manual mode depending on user requirements -it is possible to work at the frequency with which one or more species of microorganisms are subject to negative dielectrophoresis; thus it is possible to trap the aforementioned biological objects into the dielectrophoretic potential cages and move them in longer or shorter paths around the device. The proposed device has the novel feature of moving the particles in suspension within the liquid instead of moving the liquid itself, thus reducing the need for complex and expensive fluidics procedures, enabling selected bodies to accumulate in proper sites or chambers and preventing the particles from being stressed by friction and collision. During the modes of operation described so far, the embedded sensors can monitor the presence of particles, thus providing for adaptive control of the device and its functionality in a feedback loop.

One important operation the device can perform is to characterize a sample of particulate and solubilized matter by differences in the physical properties of either the population or its components. This can be achieved by using the feature of guided cages, the mobility and strength of which depend on the physical properties and morphology of the biological matter being analyzed such as size, weight,

5 polarizability and conductivity, which will vary from species to species.

10 With its unique feature of inducing independent movement of one or more particles trapped in potential cages along guide paths, the device may easily be programmed to achieve several tasks: e.g. to separate one kind of microorganism from a mixture of species by using their physical, dielectric and conductive properties. 15 Another possible application of the proposed device consists of making two or more microorganisms collide by first trapping the objects in different cages and then moving them towards the same location of the device. As an example of the wide range of application afforded by the device according to the present invention, various different 20 methods for manipulating particles are hereinafter disclosed, though again with the proviso that examples used herein are not intended as limiting the spirit of the invention.

25 It is envisioned that alternate or equivalent configurations of the present invention may be adopted without any restriction of the general invention as portrayed. Finally, it is intended that both materials and dimensions may be varied according 30 to the user or device application requirements.

35 **Method for separating particles of different types by difference in dielectrophoretic forces**

40 It is assumed that the sample in the device chamber contains a mixture of particles of at least two different types which are subject to negative dielectrophoresis and positive dielectrophoresis respectively, at a given frequency. By energizing the electrodes with periodic signals at that frequency, potential cages are established, into 45 which the particles of the first type are attracted and from which the particles of the second type are repelled. Hence by moving the potential cages toward a separate area of the device only the particle of the first type will be displaced. That area may be, for example, a separate chamber in the device where particles of the first. 50

5 type may be further collected, counted, mated with other particles etc.. It should be noted that in this case more than one particle per cage may be allowed.

10 **Method for separating particles of different types by single-particle entrapment, type detection and motion**

15 It is assumed that the sample in the device chamber contains a mixture of particles of at least two different types. It is further assumed that the size of the cages is such that only one particle may be trapped in each cage, and that each location on which the cages are established comprises a sensor able to detect the type of
20 particle trapped in that cage, if any. This sensor may, for example, be of capacitive and/or optical type. After establishment of the dielectrophoretic potential cages, the particles in each cage are discriminated, and all cages trapping particles of one type are moved toward a separate area of the device so that only particles of that type will be present in that area. That area may be a separate chamber in the device where the particles may be further collected, counted, mated with each other or with other
30 particles etc.. As used herein and in what follows, the term 'type' should be seen as referring to characteristics which may be discriminated by using sensors. In other terms, two particles made of the same matter, but of different size, may be regarded as belonging to different types if the sensor embedded in the device discriminates the two. Again, two particles made of different matter, but which cause the same output of the embedded sensor, may be regarded as belonging to the same type.

40 **Method for separating particles of different types by single-particle entrapment, motion, type detection, and motion**

45 This method is similar to the previous one, except for the fact that the locations on which the cages are first established need not comprise a sensor. Thus it is first necessary to displace particles -by moving cages -toward locations where a sensor is
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5 able to detect their type, and then further displace the particles, according to their
type, toward different areas of the device. These areas may be, for example, separate
10 chambers in the device where the particles may be further collected, counted, mated
with each other or with other particles, etc..

15 **Method for counting particles of a type by single-type of particles entrap-
ment and number detection**

It is assumed that the sample in the device chamber contains a single type of particle,
and that each location on which the cages are established comprises a sensor which is
20 able to detect the number of particles trapped in that cage. This can be achieved if
the output response of the sensor is proportional to the number of particles trapped
in the cage associated. The total number of particles in the sample can be counted
25 quite simply by summing the number of particles detected in each cage.

30 **Method for counting particles of different types by single-particle entrap-
ment and type detection**

It is assumed that the sample in the device chamber contains one or more types
of particle. It is further assumed that the size of the cages is such that only one
35 particle may be trapped in each cage, and that each location on which the cages are
established comprises a sensor able to detect the presence and type of the particle
trapped in that cage, if any. Counting the number of particles of each type can thus
40 be simply achieved by establishing potential cages, detecting the type of particle in
each cage, if any, and separately summing the number of cages trapping particles of
the same type.

5 **Method for counting particles of different types by single-particle entrapment, motion and type detection**

10 This method is similar to the previous one, except for the fact that the locations on which the cages are first established need not to comprise a sensor. Thus, it is first necessary to displace particles, by moving cages, toward locations where a
15 sensor is able to detect their type. Then the type of any particle present in the cages at the sensing locations is detected. If other cages whose content has not yet been monitored are left over, the cage at the sensing location is displaced to allow cages
20 whose content has not yet been detected to be displaced above the same sensing location. This last operation is repeated until the content of all the cages has been detected. Counting the number of particles of each type can therefore be achieved
25 by separately summing the number of cages trapping particles of the same type.

Claims

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CLAIMS:

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1. Apparatus for the manipulation of one or more particles immersed in a fluid, comprising:

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- a first substrate;
- a plurality of electrodes comprising a first electrode array realized on said first substrate and a second electrode array comprising at least one electrode, said second electrode array being faced toward and spaced apart from said first electrode array, said particles and said fluid being placed in a region between said first electrode array and said second electrode array;

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- means for applying a first electrical input to a first subset of said plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes

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said first electrical input and said at least one other electrical input establishing an electric-field having constant magnitude over at least one imaginary closed surface located entirely in said fluid.

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2. Apparatus according to claim 1, wherein said second electrode array is realized on a second substrate.

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3. Apparatus according to claim 1, wherein said first substrate includes sensing means for detecting the presence of one or more of said particles.

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4. Apparatus according to claim 2, wherein said first substrate and/or second substrate includes sensing means for detecting the presence of one or more of said particles.

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5. Apparatus according to claim 3 or 4, wherein said sensing means include electric-field measuring means for detecting variations in the electrical characteristics in at least a portion of said region between said first electrode array and said second electrode array.
 6. Apparatus according to claim 5, wherein said electric-field measuring means include at least one electrode of said second electrode array and at least one electrode of said first electrode array, for detecting variations in the electric-field established between said at least one electrode of said second electrode array and said at least one electrode of said first electrode array.
 7. Apparatus according to claim 5, wherein said electric-field measuring means include a first electrode of said first electrode array and at least one other electrode of said first electrode array, for detecting variations in the electric-field established between said first electrode and said at least one other electrode.
 8. Apparatus according to claim 1, wherein said second electrode array is substantially transparent.
 9. Apparatus according to claim 3 and 8, wherein said sensing means include optical-energy measuring means for detecting variations in the optical characteristics in at least a portion of said region between said first electrode array and said second electrode array.
 10. Apparatus according to any one of the previous claims, further comprising means for changing said first electrical input and/or said at least one other electrical input for:
 - expanding or contracting, and/or
 - moving, and/or

5 - establishing or deleting

 said at least one imaginary closed surface

10 11. Apparatus according to any one of the previous claims, further comprising
 means for changing said first and/or said at least one other subset of said
 plurality of electrodes for:

15 - expanding or contracting, and/or

 - moving, and/or

20 - establishing or deleting

 said at least one imaginary closed surface.

25 12. Apparatus according to any one of the previous claims, further comprising
 means to let said fluid flow into and/or out of said region between said first
 electrode array and said second electrode array.

30 13. Apparatus according to any one of the previous claims, further comprising a
 spacer interposed between said first substrate and said second electrode ar-
 ray, said spacer having at least one opening, said spacer forming at least one
35 chamber between said first substrate and said second electrode array.

40 14. Apparatus according to any one of claims 1-12, further comprising a spacer
 integrated in said first substrate, said spacer having at least one opening, said
 spacer forming at least one chamber between said first substrate and said sec-
 ond electrode array.

45 15. Apparatus according to claim 2 or 4, further comprising a spacer integrated in
 said first substrate and/or said second substrate, said spacer having at least one
 opening, said spacer forming at least one chamber between said first substrate
50 and said second electrode array.

5 16. Apparatus according to any one of the previous claims, wherein at least one
electrode of said plurality of electrodes is connected to circuit means compris-
10 ing:

- 15 - addressing input means;
- data input/output means;
- reference input means;
- at least one memory element;

20 whereby the electrical input applied to the electrode is derived from said refer-
ence input according to a value stored in said at least one memory element pro-
grammed by said addressing input means and said data input/output means.

25 17. Apparatus according to claim 16, wherein said circuit means further comprises
sensing means.

30 18. Apparatus according to any one of the previous claims, wherein at least one of
said electrodes of said first electrode array has rectangular shape.

35 19. Apparatus according to any one of the previous claims, wherein at least one of
said electrodes of said first electrode array has hexagonal shape.

40 20. Apparatus according to any one of the previous claims, wherein said second
electrode array consists of a single electrode.

45 21. Apparatus according to any one of the previous claims, wherein said first sub-
strate is a monolithic semiconductor substrate.

50 22. Method for the manipulation of one or more particles immersed in a fluid,
applying a first electrical input to a first subset of a plurality of electrodes and
at least one other electrical input to at least one other subset of said plurality
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5 of electrodes, in order to establish an electric field having constant magnitude
over at least one imaginary closed surface located entirely in said fluid, whereby
10 said particles are either attracted toward or repelled from the region enclosed
by said at least one imaginary closed surface depending on electrical properties
of said particles and said fluid.

15 23. Method for the manipulation of one or more particles immersed in a fluid,
comprising the steps of:

- 20 - applying a first electrical input to a first subset of a plurality of electrodes
and at least one other electrical input to at least one other subset of said
plurality of electrodes in order to establish an electric field having constant
25 magnitude over at least one imaginary closed surface located entirely in
said fluid, whereby said particles are attracted toward the region enclosed
by said at least one imaginary closed surface ;
- 30 - displacing said at least one imaginary closed surface in order to attract
said particles toward another region enclosed by said at least one imagi-
nary closed surface .

35 24. The method of claim 23 wherein said displacing of said at least one imaginary
closed surface is obtained by changing at least once said first subset of the
plurality of electrodes and/or said at least one other subset of the plurality of
40 electrodes.

45 25. The method of claim 23 wherein said displacing of said at least one imaginary
closed surface is obtained by changing said first electrical input and/or said at
least one other electrical input.

50 26. Method for the separation of different types of particles immersed in a fluid,
comprising the steps of:

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- applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having constant magnitude over at least one imaginary closed surface located entirely in said fluid, whereby the particles of at least one first type are attracted toward the region enclosed by said at least one imaginary closed surface and the particles of different types are repelled from said region;
 - displacing said at least one imaginary closed surface in order to move only said particles of at least one first type toward another region enclosed by said at least one imaginary closed surface.

25 27. Method for the separation of different types of particles immersed in a fluid, comprising the steps of:

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- applying a first electrical input to a first subset of a plurality of electrodes and at least one other electrical input to at least one other subset of said plurality of electrodes, in order to establish an electric field having constant magnitude over multiple imaginary closed surfaces located entirely in said fluid, whereby said different types of particles are attracted toward and trapped in the regions enclosed by said imaginary closed surfaces, each of said regions being able to trap only one particle;
 - sensing the type of each particle trapped in said regions;
 - displacing a first subset of said imaginary closed surfaces toward a first area, said first subset being composed of imaginary closed surfaces which trap particles of a first type, in order to move said particles of a first type toward said first area.

5
28. Method for the separation of different types of particles immersed in a fluid,
comprising the steps of:

- 10 - applying a first electrical input to a first subset of a plurality of electrodes
 and at least one other electrical input to at least one other subset of said
 plurality of electrodes, in order to establish an electric field having con-
15 stant magnitude over multiple imaginary closed surfaces located entirely
 in said fluid, whereby said different types of particles are attracted to-
 ward the regions enclosed by said imaginary closed surfaces, each of said
20 imaginary closed surfaces being able to trap only one particle;
- sequentially displacing said imaginary closed surfaces toward at least one
 sensing location, in order to move trapped particles toward said at least
25 one sensing location, and sensing the type of each particle in each of said
 at least one sensing location;
- displacing toward a first area a first subset of said imaginary closed sur-
30 faces composed of those imaginary closed surfaces which trap particles of
 a first type, in order to move said particles of a first type toward said
 first area, and displacing toward a second area a second subset of said
35 imaginary closed surfaces composed of imaginary closed surfaces which
 trap particles of different types, in order to move the particles of different
 types toward said second area.

40
29. Method for counting the number of particles of at least one type immersed in
a fluid, comprising the steps of:

- 45 - applying a first electrical input to a first subset of a plurality of electrodes
 and at least one other electrical input to at least one other subset of
 said plurality of electrodes, in order to establish an electric field having
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5 constant magnitude over at least one imaginary closed surface located
entirely in said fluid, whereby only said particles of at least one type are
10 attracted toward the regions enclosed by said at least one imaginary closed
surface;

- sensing the number of particles in each of said regions.

15 30. Method for counting the number of particles of at least one type immersed in
a fluid, comprising the steps of:

20 - applying a first electrical input to a first subset of a plurality of electrodes
and at least one other electrical input to at least one other subset of said
plurality of electrodes, in order to establish an electric field having con-
stant magnitude over at least one imaginary closed surface located entirely
25 in said fluid, whereby said particles are attracted toward the regions en-
closed by said at least one imaginary closed surface, each of said regions
being able to trap only one particle;

30 - sensing the presence and type of each particle in said regions;

- separately summing the number of particles of the same type.

35 31. Method for counting the number of particles of at least one type immersed in
a fluid, comprising the steps of:

40 - applying a first electrical input to a first subset of a plurality of electrodes
and at least one other electrical input to at least one other subset of said
plurality of electrodes, in order to establish an electric field having con-
stant magnitude over multiple imaginary closed surfaces located entirely
45 in said fluid, whereby said particles are attracted toward the regions en-
closed by said imaginary closed surfaces, each of said regions being able
to trap only one particle;

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5 -- sequentially displacing said imaginary closed surfaces toward at least one
sensing location, in order to move the trapped particles toward said at
least one sensing location, sensing the presence and type of each particle
10 in each of said at least one sensing location;

-- separately summing the number of particles of the same type.

15 32. The method of any one of claims 26, 27, 28, 31 wherein said displacing of
the imaginary closed surfaces is obtained by changing said first subset of the
plurality of electrodes and/or said at least one other subset of the plurality of
20 electrodes.

25 33. The method of any one of claims 27, 28, 29, 30, 31, wherein the detection is
obtained by measuring variations in the electrical and/or optical characteristics
in at least a portion of said fluid.

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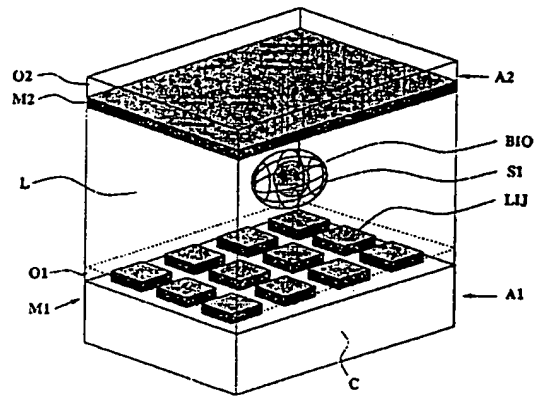


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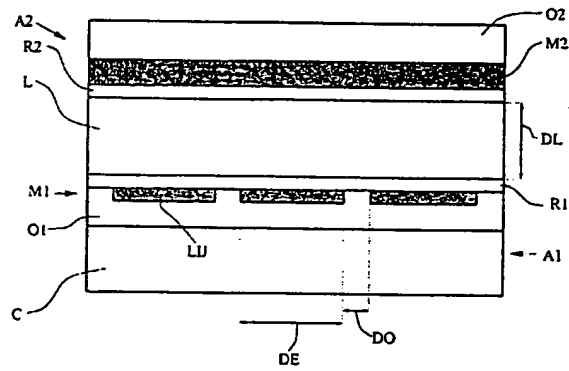


Figure 2:

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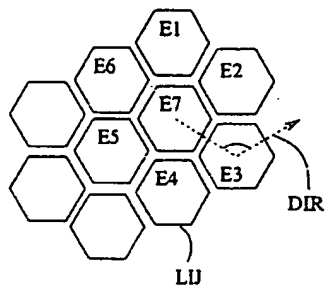


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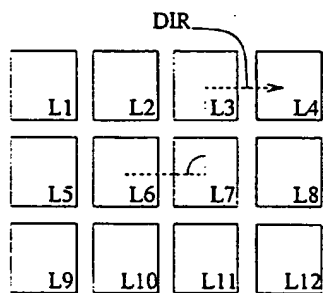


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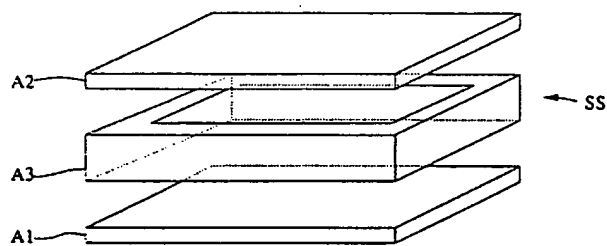


Figure 5:

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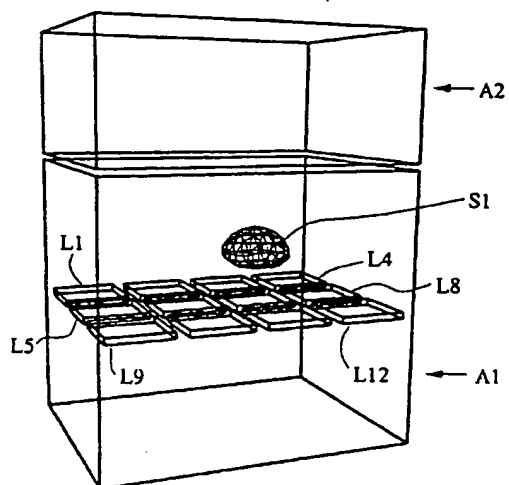


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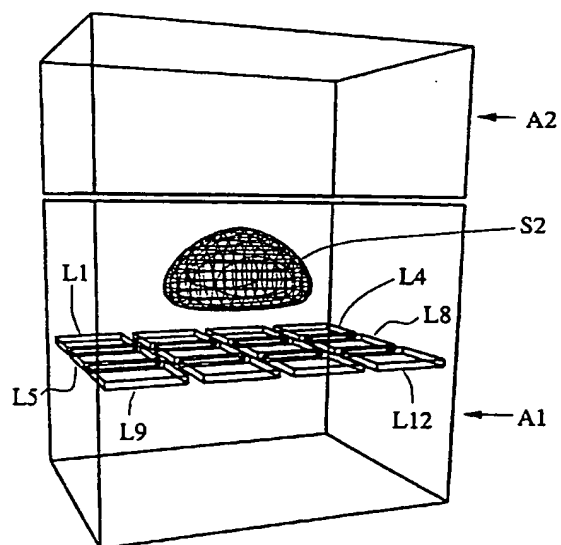


Figure 7:

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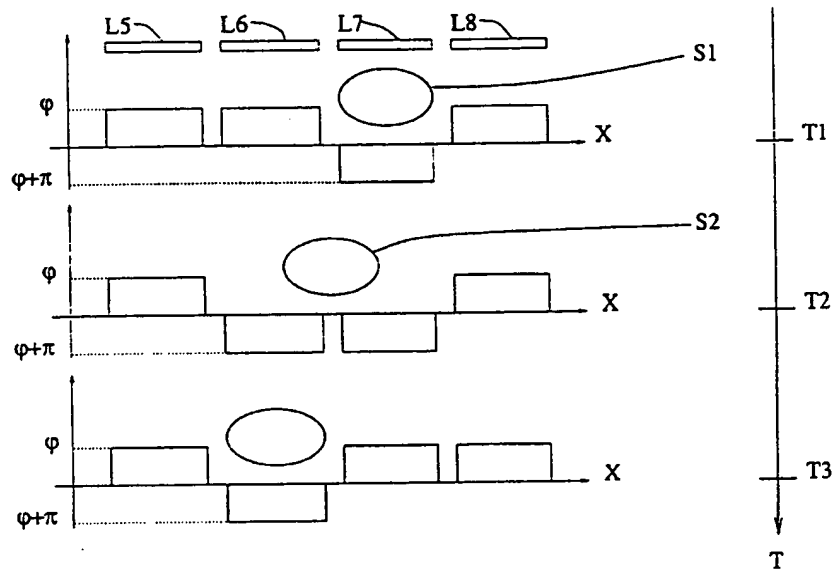


Figure 8:

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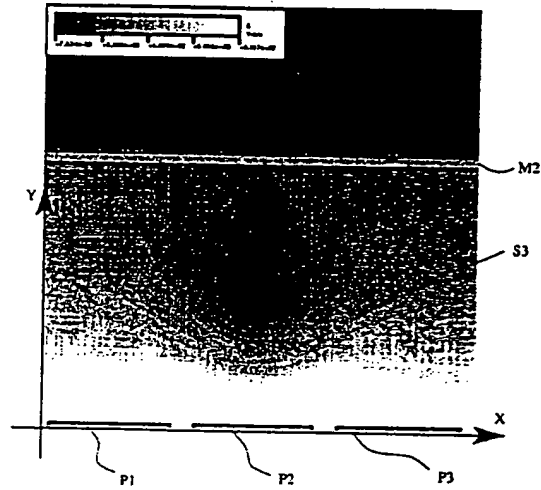


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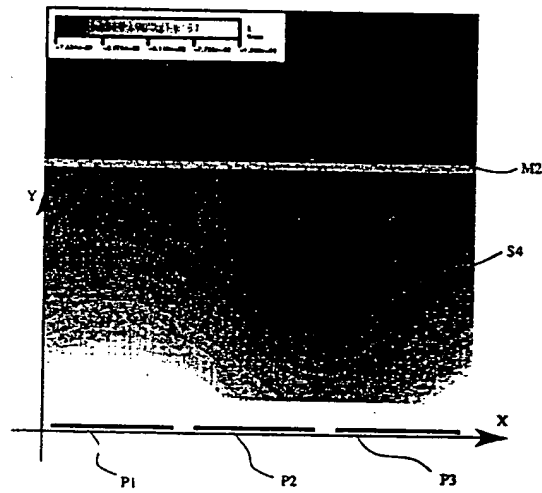


Figure 10:

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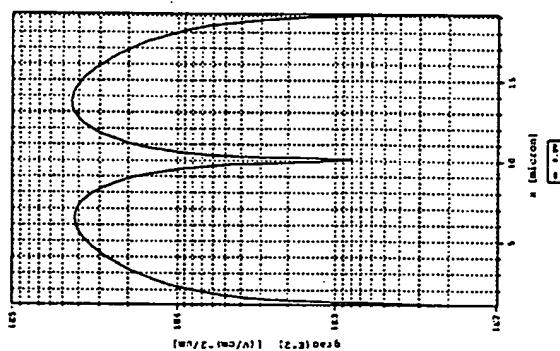


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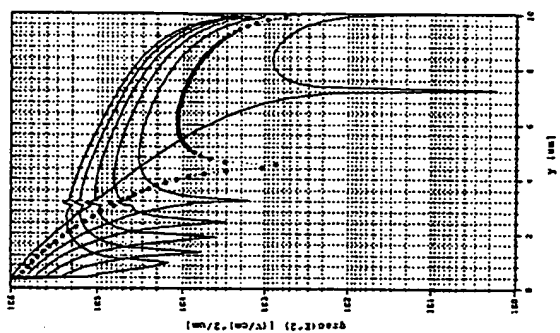


Figure 12:

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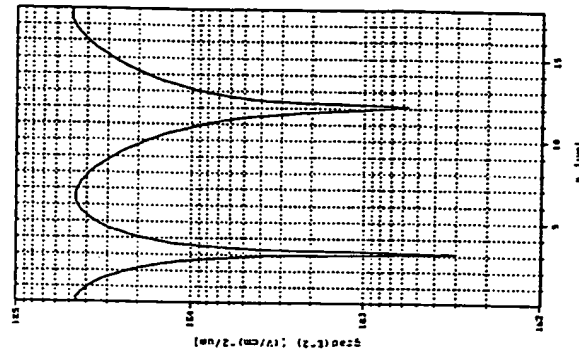


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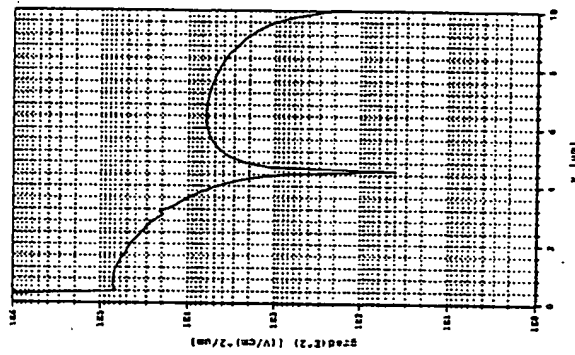


Figure 14:

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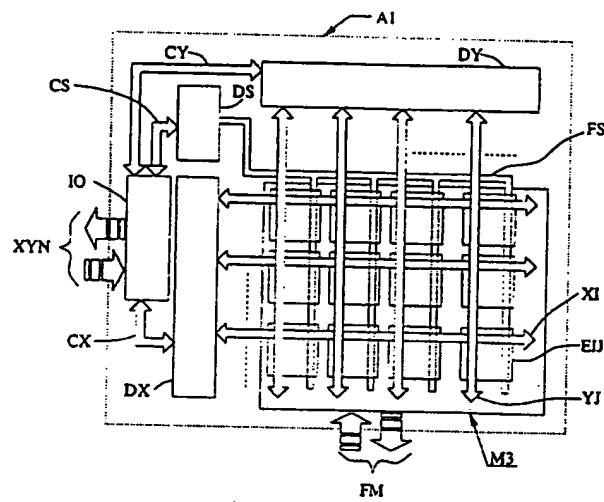


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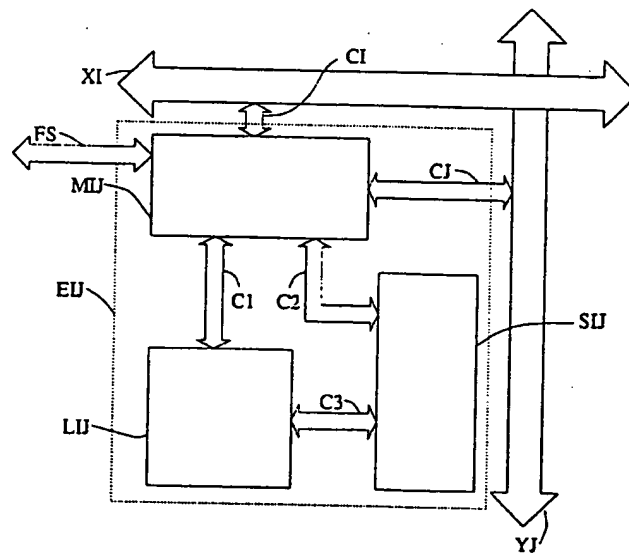


Figure 16:

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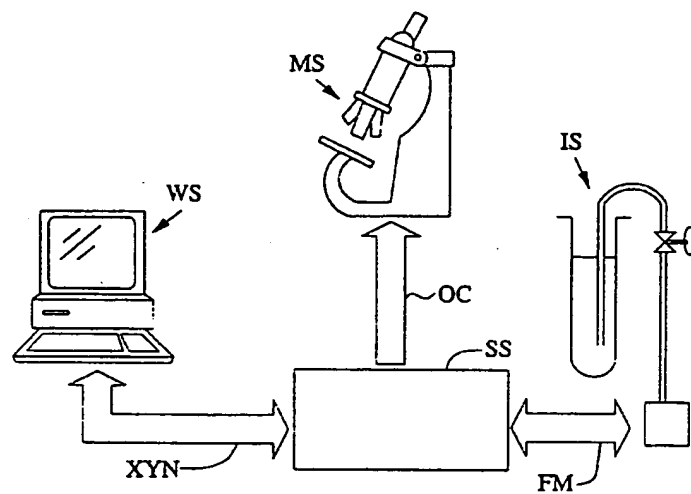


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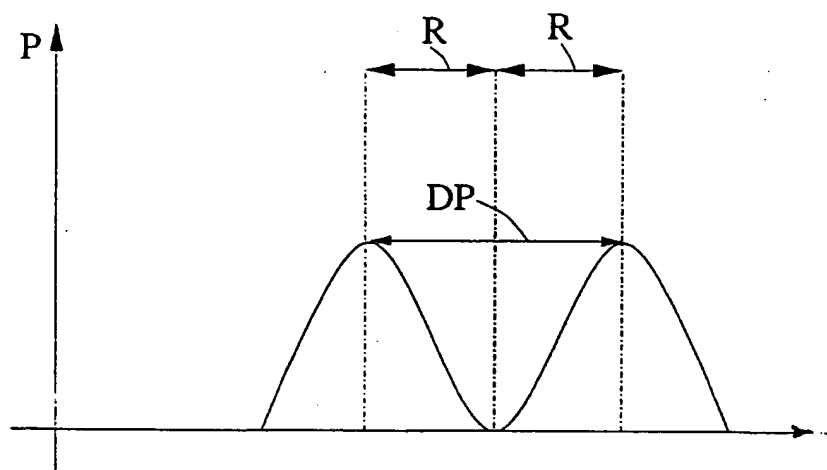


Figure 18:

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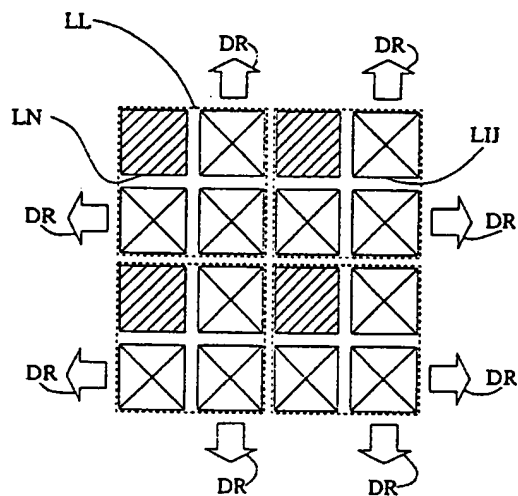


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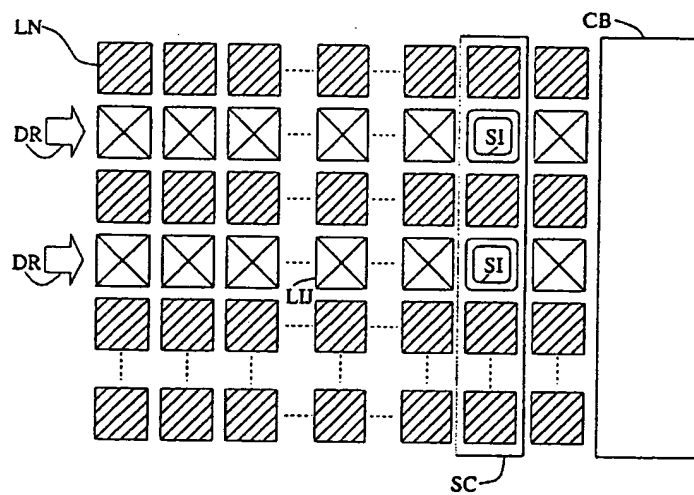


Figure 20:

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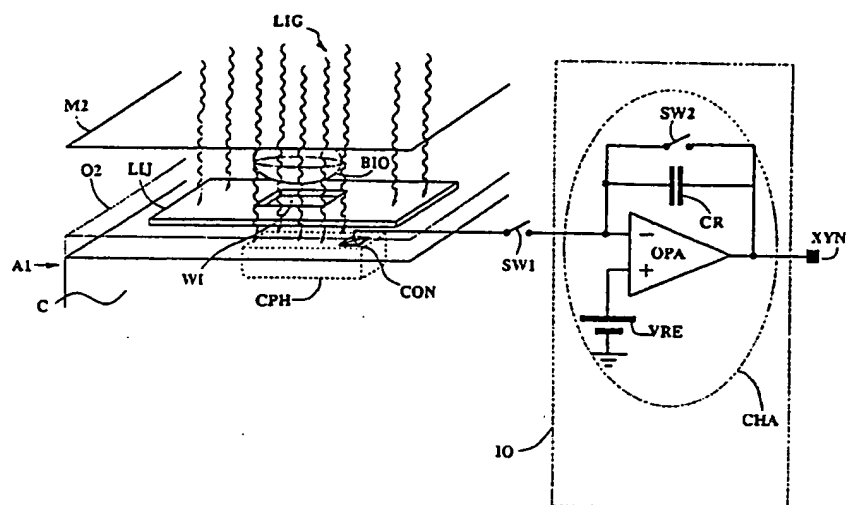


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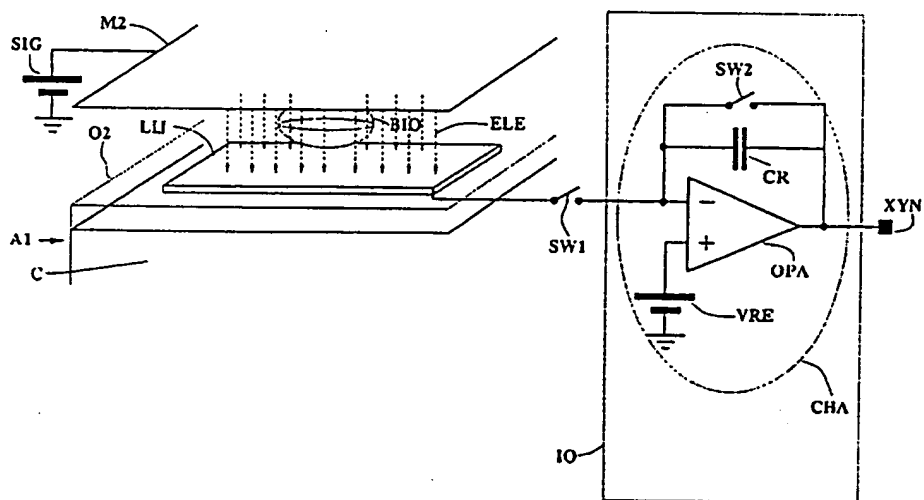


Figure 22:

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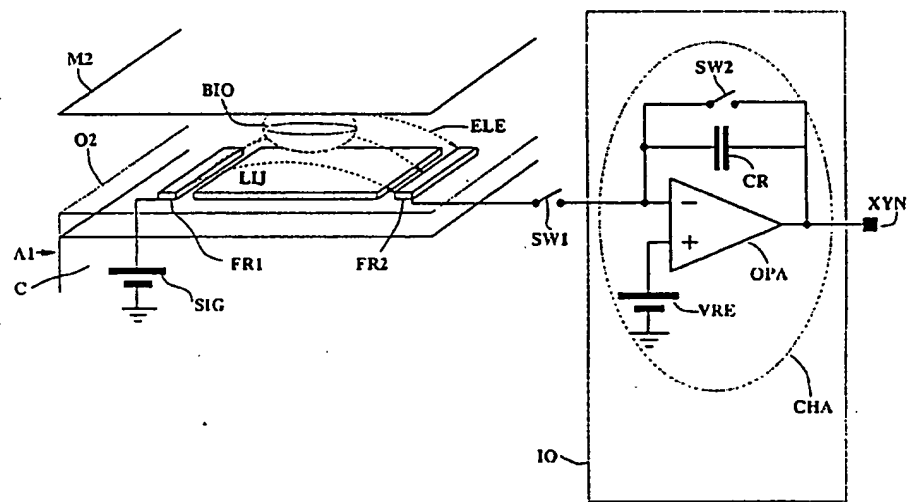


Figure 23:

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IB 00/00641

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 803C5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 803C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>FUHR G ET AL: "POSITIONING AND MANIPULATION OF CELLS AND MICROPARTICLES USING MINIATURIZED ELECTRIC FIELD TRAPS AND TRAVELLING WAVES" SENSORS AND MATERIALS, JP, SCIENTIFIC PUBLISHING DIVISION OF MYU, TOKYO, vol. 7, no. 2, 1995, pages 131-146, XP000617993 ISSN: 0914-4935 page 131 -page 134, paragraph 1; figure 4</p> <p style="text-align: center;">-/--</p>	<p>1,2,10, 12-15, 21-23,25</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

15 August 2000

Date of mailing of the international search report

23/08/2000

Name and mailing address of the ISA

European Patent Office, P.O. 5818 Patentaan 2
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Fax: (+31-70) 340-3016

Authorized officer

Decanniere, L

INTERNATIONAL SEARCH REPORT

Int'l. Patent Application No
PCT/IB 00/00641

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SCHNELLE T ET AL: "THREE-DIMENSIONAL ELECTRIC FIELD TRAPS FOR MANIPULATION OF CELLS - CALCULATION AND EXPERIMENTAL VERIFICATION"</p> <p>BBA - GENERAL SUBJECTS, NL, ELSEVIER SCIENCE PUBLISHERS,</p> <p>vol. 1157, no. 2, 1993, pages 127-140,</p> <p>XP000614981</p> <p>ISSN: 0304-4165</p> <p>cited in the application</p> <p>the whole document</p>	<p>1,2,8,</p> <p>12,13,</p> <p>16,18,</p> <p>22,26.</p>
A	<p>WO 99 17883 A (CALIFORNIA INST OF TECHN)</p> <p>15 April 1999 (1999-04-15)</p> <p>claims 1,3-5,9,33,34</p>	<p>1,2,10,</p> <p>22,23,25</p>
A	<p>FIEDLER S ET AL: "ELECTROCASTING - FORMATION AND STRUCTURING OF SUSPENDED MICROBODIES USING A.C. GENERATED FIELD CAGES"</p> <p>MICROSYSTEM TECHNOLOGIES, DE, BERLIN,</p> <p>1 December 1995 (1995-12-01), pages 1-7,</p> <p>XP000199394</p> <p>ISSN: 0946-7076</p> <p>figure 2</p>	<p>1,8,9</p>
A	<p>WO 91 08284 A (NAT RES DEV)</p> <p>13 June 1991 (1991-06-13)</p> <p>claims 1,3,5</p>	<p>1,8,9</p>

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No
PCT/IB 00/00641

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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